Increased sensorimotor gating in recreational and dependent cocaine users is modulated by craving and attention-deficit/hyperactivity disorder symptoms

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Abstract: BACKGROUND: Cocaine dependence has been associated with blunted dopamine and norepinephrine signaling, but it is unknown if recreational cocaine use is also associated with alterations of catecholamine systems. Prepulse inhibition (PPI) of the acoustic startle response-a measure of sensorimotor gating-is highly sensitive for manipulations of the catecholamine system. Therefore, we investigated whether relatively pure recreational users (RCU) and dependent cocaine users (DCU) display alterations of PPI, startle reactivity, and habituation. Moreover, the influences of methylenedioxymethamphetamine and cannabis co-use, craving, and attention-deficit/hyperactivity disorder (ADHD) symptoms on startle measures were examined. METHODS: In 64 RCU, 29 DCU, and 66 stimulant-naïve control subjects, PPI of acoustic startle response, startle reactivity, habituation, ADHD symptoms, and cocaine craving were assessed. Drug use of all participants was controlled by hair and urine toxicologies. RESULTS: Both RCU and DCU showed increased PPI in comparison with control participants (Cohen's d=.38 and d=.67, respectively), while RCU and DCU did not differ in PPI measures (d=.12). No significant group differences were found in startle reactivity or habituation measures. In cocaine users, PPI was positively correlated with cumulative cocaine dose used, craving for cocaine, and ADHD symptoms. Users with a diagnosis of ADHD and strong craving symptoms displayed the highest PPI levels compared with control subjects (d=.78). CONCLUSIONS: The augmented PPI in RCU and DCU suggests that recreational use of cocaine is associated with altered catecholamine signaling, in particular if ADHD or craving symptoms are present. Finally, ADHD might be a critical risk factor for cocaine-induced changes of the catecholamine system.

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Increased sensorimotor gating in recreational and dependent cocaine users is modulated by craving and ADHD symptoms

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Abstract

Background: Cocaine dependence has been associated with blunted dopamine and norepinephrine signaling but it is unknown if also recreational cocaine use is associated with alterations of catecholamine systems. Prepulse inhibition (PPI) of the acoustic startle response (ASR) – a measure of sensorimotor gating – is highly sensitive for manipulations of the catecholamine systems. Therefore, we investigated whether relatively pure recreational (RCU) and dependent cocaine users (DCU) display alterations of PPI, startle reactivity, and habituation. Moreover, the influences of MDMA and cannabis co-use, craving, and Attention-Deficit/Hyperactivity Disorder (ADHD) symptoms on startle measures were examined.

Methods: In 64 RCU, 29 DCU, and 66 stimulant-naïve controls PPI of ASR, startle reactivity, habituation, ADHD symptoms, and cocaine craving were assessed. Drug use of all participants was controlled by hair and urine toxicologies.

Results: Both, RCU and DCU showed increased PPI in comparison to control participants (Cohen's *d*=.38, *d*=.67, respectively), while RCU and DCU did not differ in PPI measures (*d*=.12). No significant group differences were found in startle reactivity or habituation measures. In cocaine users, PPI was positively correlated with cumulative cocaine dose used, craving for cocaine, and ADHD symptoms. Users with a diagnosis of ADHD and strong craving symptoms displayed the highest PPI levels compared to controls (*d*=.78).

Conclusion: The augmented PPI in RCU and DCU suggests that already recreational use of cocaine is associated with altered catecholamine signaling in particular if ADHD or craving symptoms are present. Finally, ADHD might be a critical risk factor for cocaine-induced changes of the catecholamine system.

Introduction

Cocaine is an illegal drug with a high tendency to induce dependence when chronically abused (1). Nevertheless, a substantial part of people use cocaine in a recreational and non-dependent manner (2). The lifetime prevalence of cocaine use is estimated at 5.9% amongst 15-34 year olds in Europe. It has been established as the most commonly stimulant drug used in Europe (2) and is the primary stimulant drug responsible for drug-dependence treatment in North and South America (3).

Cocaine inhibits the reuptake of dopamine (DA), norepinephrine (NE), and serotonin (4). Marked structural and functional alterations in striatal and prefrontal regions have been reported in dependent cocaine users (DCU)(5-9). Furthermore, reduced striatal DA D2 receptor availability and blunted striatal DA release have repeatedly been shown to be associated with chronic cocaine use in humans using PET imaging (10-13). Striatal DA is a central mediator of reward, memory, and behavioral inhibition (14). Thus, dysregulated DA functioning in DCU has been associated with widespread consequences including craving, impulsive behavior, loss of control over drug intake, and relapse (12,13,15). Recently, also upregulation of thalamic NE transporters has been reported in DCU (16) and NE seems to play an important role in craving, withdrawal-related anxiety, and relapse in cocaine addiction (17,18). While most studies investigated dependent users, little is known about the effects of occasional and recreational cocaine use. However, a recent study showing blue-yellow color vision deficits in recreational cocaine users (RCU) and DCU suggests that already recreational cocaine use might lead to changes in DA function (19).

Prepulse inhibition (PPI) of the acoustic startle response (ASR) refers to the attenuation of the reflexive startle reaction when the startling stimulus is preceded 30-500ms by a weak and non-startling stimulus (20). PPI is considered as a translational measure of sensorimotor gating, reflecting a universal pre-attentional filter function (21) that is regulated by a cortico-striato-pallido-pontine (CSPP) circuit involving the prefrontal cortex, the ventral striatum including NAcc, the ventral pallidum, and the pontine tegmentum (22). PPI has been shown to be highly sensitive to changes in catecholamine neurotransmission, especially in the ventral part of the mesostriatal DA system (23,24), and in thalamocortical and ventral forebrain NE networks (25-28).

Acute administration of cocaine reduced PPI in rats (29), but lasting effects of repeated drug exposure are less well studied. In cocaine-withdrawn rats no alterations in PPI were found (29,30). In humans, Efferen et al.(31) reported a trend towards increased PPI in a small sample of DCU (n=10 vs. n=9

controls). In this study, DCU displayed reduced startle reactivity (31), while a subsequent study revealed that decreased startle was only present in DCU with continued abstinence (>40 days) (32). With regard to startle reactivity, animal results are inconsistent. Adams et al.(30) showed reduced startle reactivity in cocaine-withdrawn rats, whereas Martinez et al.(29) found no significant differences in startle reactivity or habituation. However, PPI has not been studied in a sufficiently large sample of cocaine users so far. Moreover, the effects of recreational cocaine use on PPI and startle reactivity are unknown. The overlap of reward circuits shown to be altered in cocaine users (in particular the ventral striatum) and CSPP circuits regulating PPI suggests that PPI may be altered in cocaine addiction (9,10,22,23).

Therefore, we aimed to investigate startle reactivity and PPI in large groups of RCU and DCU, and stimulant-naïve control subjects. Preliminary data from a previous study predict increased PPI levels and decreased ASR in DCU (31), while we expect to find a similar but less pronounced pattern already in RCU (19). Given that Attention-Deficit/Hyperactivity Disorder (ADHD) is highly comorbid with cocaine dependence and abuse (33) and it was shown that ADHD patients also display alterations in DA and NE signaling (34,35), we additionally assessed the severity of ADHD symptoms in our subjects. Moreover, we examined the severity of cocaine craving, as craving was associated with low striatal DA levels and NE alterations as well (12,36). Psychiatric comorbidities, such as ADHD, and polytoxic drug use have been shown to be confounded in studies especially with DCU (37,38). We aimed to overcome these previous limitations I) by the inclusion of a group of recreational and presumably less comorbid and polytoxic users, II) by controlling ADHD symptoms, and III) by the application of comprehensive psychiatric diagnostics and the examination of hair toxicologies allowing the exclusion of subjects with psychiatric diseases (other than substance dependence or ADHD) and polytoxic drug use patterns.

Methods

Participants

Twenty-nine DCU, 64 RCU, and 66 drug-naïve control participants took part in the study (for recruitment and selection details see **supplemental information**). Cocaine dependence was diagnosed following the Diagnostic and Statistical Manual-IV (DSM-IV) criteria (39), with DCU fulfilling these criteria and RCU not meeting dependency criteria. Further inclusion criteria for the two user groups were cocaine use of at least 1g/month, cocaine as primary used illegal drug, and a current abstinence duration <6 months. Participants had to be aged between 18 and 60 years. Exclusion criteria for the user groups were use of opioids, a polytoxic drug use pattern, and an Axis-I DSM-IV adult psychiatric disorder with exception of cocaine and alcohol abuse/dependence, a history of a depression (acute major depression was excluded), and ADHD. Control subjects were excluded when they displayed any Axis-I DSM-IV psychiatric disorder inclusive ADHD and any form of addiction or regular illegal drug use (lifetime use <15 occasions) with exception of cannabis. Exclusion criteria for all participants were a neurological disorder or head injury, clinically relevant medical diseases, family history of schizophrenia or bipolar disorder, or prescription drugs affecting the CNS, and medical conditions concerning eyes, ears, and equilibrium organs. All participants had to abstain from illegal substances for a minimum of three days and from alcohol for at least 24h. Self-reports were controlled by urine and 6-month hair analysis (details see **supplemental information**).

The study was approved by the Cantonal Ethics Committee of Zurich (KEK). All participants provided written informed-consent and were compensated for their participation.

Procedure

The present data were collected as part of a larger longitudinal study on socio-cognitive consequences of cocaine use – the Zurich Cocaine Cognition Study ($ZuCo^2St$). A Structured Clinical Interview for DSM-IV Disorders (SCID-I) was carried out by a trained psychologist. Drug use was assessed by means of the Interview for Psychotropic Drug Consumption (40). The brief version of the Cocaine Craving Questionnaire (CCQ)(41) was applied to assess current cocaine craving. The Fagerström Test of Nicotine Dependence (FTND)(42) was used to assess the level of nicotine dependency. The

Mehrfachwahl-Wortschatz-Intelligenztest (MWT-B)(43), a standardized German vocabulary test, was carried out for the estimation of premorbid verbal intelligence quotient (IQ). The Beck Depression Inventory (BDI)(44) measured the current severity of depressive symptoms, and the ADHD self-rating scale (ADHD-SR)(45) was applied to allow for the diagnosis of ADHD in adulthood according to DSM-IV criteria. A neuropsychological test battery was assessed (data will be published elsewhere) and subsequently the ASR measurement was conducted. Participants had to abstain from smoking for at least 60min prior to startle testing.

Startle Response Measurement

For a detailed description of the paradigm, see **supplemental information**. In brief, startle stimuli comprised of noise bursts at an 115dB sound pressure level with duration of 40ms, separated by variable inter-trial-intervals (range: 9-14 seconds, mean: 12 seconds). After an initial pulse-alone trial (PA) the session included a total of 64 trials (56 active and 8 no-stimulation trials). Thirty-two pulse trials were preceded by a 20ms prepulse with an intensity of 86dB and a stimulus onset asynchrony (SOA) of 30, 60, 120, and 240ms, resulting in four SOA conditions. The eye-blink component of the ASR was measured by an EMG startle system (EMG-SR-Lab; San Diego Instruments, USA) as described previously (21). Preprocessing of the recorded data was performed using Analyzer software (Brainvision, Germany) and emgBLINK version 1.2 (CST, Switzerland) as described in detail previously (46).

Statistical Analysis

Frequency data were analyzed by means of Pearson's Chi-square test and quantitative data by analyses of variance (ANOVA) using PASW 18.0 (IBM, Switzerland). PPI and habituation data were normally distributed in each group (Kolmogorov- Smirnov test $p>.05$) and were therefore analyzed parametrically. The mean %PPI was calculated for each SOA as described prevously (21,47). These data were analyzed using mixed-design analyses of covariance (ANCOVA) with Greenhouse-Geisser corrections, followed by Sidak-corrected pair-wise comparisons and simple main effects analyses. SOA condition was introduced as within-subjects factor and group as between-subjects factor. As

smoking status, sex (3-fold: women differentiated by luteal vs. follicular phase of the menstrual cycle), and age were shown to influence startle parameters (48-52), these variables were introduced as covariates.

Startle reactivity measures were analyzed likewise. To assess habituation, PA trials of block 1-4 were analyzed conducting a mixed-design ANCOVA (53). Percent habituation was calculated as the reduction in startle magnitude between the second block and following blocks of PA trials (%Habituation=100*(block 2–block n)/block 2) to avoid sensitization effects (27). Furthermore, the linear gradient coefficient *b* was calculated across the four blocks of PA as described previously (47). %Habituation and *b* were analyzed by one-way ANCOVAs controlling for smoking status, sex, and age.

Correlation analyses (Pearson's product-moment) were conducted to relate drug use parameters to PPI measures. Cumulated cocaine lifetime use was ln-transformed for statistical analyses because of the highly skewed distribution and the resulting deviation from the normal distribution (Shapiro-Wilk $W<.001$ for both user groups). For correlations between illegal drug use and PPI control subjects were excluded to prevent inflating existing correlations. The confirmatory statistical comparisons of all data were carried out on a significance level set at $p<.05$ (two-tailed).

Results

Demographic characteristics

The groups did not differ with respect to IQ, sex distribution, and proportion of smokers and non-smokers (**Table 1**). DCU were older than controls and RCU (p<.05) and had fewer years of education than controls (p<.05). As expected, both user groups scored higher on the BDI and ADHD-SR sum scores compared to controls (all p<.01). FTND sum score differed between the smokers in each group (p<.05-.01). The hair samples revealed a clear dominance of cocaine compared to other illegal drugs as strived for by the inclusion criteria (**Table 2**). DCU showed a more than 7-fold higher concentration of cocaine and metabolites in the hair samples compared to RCU. However, RCU are regular users with a mean weekly consumption of about 1g cocaine but without fulfilling DSM-IV criteria for cocaine dependence. A considerable amount of participants tested positive in urine toxicologies for cocaine and cannabis and we decided not to exclude them but to investigate the acute and post-acute effects of the drugs.

PPI

A mixed effects ANCOVA (SOA*group) revealed a significant main effect for SOA ($F(3,459)=9.50$, p<.001) and a significant between subjects effect for group ($F(2,153)=4.58$, p<.01)(**Figure 1**). The main effect of SOA reflects the nature of PPI to increase with rising SOA (54). The interaction of SOA*group was not significant ($F(6,459)=.97$, p=.44). Sidak-corrected pairwise comparisons showed a significantly increased mean %PPI for RCU (p<.05, d=.38) and DCU (p<.05, d=.67) compared to controls, whereas RCU and DCU did not differ from each other (p=.94, d=.12). A significant effect was found for the covariate sex ($F(1,153)=4.95$, p<.05) (women in follicular phase and men>women in luteal phase), whereas the effects of age ($F(1,153)=1.74$, p=.19) and smoking status ($F(1,153)=.64$, p=.43) did not reach significance.

Startle Reactivity and Habituation

ANCOVAs performed for groups did not show significant differences in startle reactivity measures (all $p>.42$), although DCU showed slightly decreased startle reactivity compared to controls (d=.36, d=.43, respectively)(**Table 3**). Furthermore, no significant differences between groups were found for the mean of PPA trials and no-stimulation trials (all $p>.53$). Running the PPI analysis with and without covarying for PA first block yielded similar results.

A mixed-design ANCOVA (PA trials block 1-4*group, smoking status, sex, age, and startle reactivity in block 1 as covariates) did not reveal a significant block*group interaction ($F(6,411)=.40$, $p=.85$), indicating that habituation did not differ between groups (**Figure S1**). Furthermore, %habituation did not differ between groups across blocks and neither did the linear gradient coefficient *b* (all $p>.44$)(**Table 3**).

Correlations with cocaine use parameters

In cocaine users, %PPI mean across SOA conditions was significantly correlated with cumulated cocaine use (ln-transformed)($r=.23$, $p<.03$) and duration of cocaine use ($r=.22$, $p<.03$), indicating an increasing PPI with growing amount and time of use (**Figure S2)**. The effects remained after adjusting for age. Correlations between %PPI mean across conditions and current cocaine use, as well as hair samples did not reach significance (p>.28).

Urine toxicology and drug use

To test the influence of recent cocaine use, cocaine users were divided into users with positive (n=26, range: 268–43’219ng/ml, mean: 5’438ng/ml, SD: 10’599ng/ml) and users with negative urine samples (n=67) and compared with controls (n=66). A mixed effects ANCOVA (SOA*group) revealed a significant between subjects effect for group ($F(2,153)=4.83$, $p<.01$). Sidak-corrected pair-wise comparisons yielded a significantly increased mean %PPI in users with negative urine samples in comparison to controls ($p<.01$, $d=.50$). The SOA*group interaction did not reach significance **(Figure S3)**.

Analogously, the influence of recent cannabis use was investigated by dividing cocaine users into users with positive (n=34, range: 60–726ng/ml, mean: 120ng/ml, SD: 139ng/ml) and users with negative urine samples for cannabis (n=59). They were compared to controls with positive (n=12, range: 66–426ng/ml, mean: 163ng/ml, SD: 131ng/ml) and controls with negative urine samples (n=54). A mixed effects ANCOVA (SOA*group) revealed a significant between subjects effect for group ($F(3,152)=3.33$, $p<.02$). Sidak-corrected pair-wise comparisons yielded still significant differences between users with negative urine samples and controls with negative urine samples ($p<.01$, $d=.51$) regarding mean %PPI **(Figure 2)**. The interaction of SOA*group was not significant ($F(9,453)=0.62$, $p>.78$).

An ANCOVA (with age and sex as covariates) of mean %PPI comparing controls and cocaine users stratified for smoking status revealed a significant group effect ($F(3,153)=4.73$, $p<.01$)(**Figure 3**). Non-smoking controls differed significantly from smoking cocaine users ($p<.01$, $d=.90$), while the difference to the non-smoking cocaine users was not significant ($p<.30$) despite the considerable effect size ($d=.63$) due to the small group sizes (n=16 vs. n=19).

A detailed analysis of the influence of the use of MDMA and cannabis on PPI is presented in the **supplemental material**.

Craving and ADHD

In cocaine users, CCQ sum score correlated significantly with %PPI mean across conditions ($r=.22$, $p<.03$). Furthermore, when dividing users in a high and low craving group by median split and comparing them with controls (**Figure S4**), a mixed effects ANCOVA (SOA*group) revealed a significant main effect for group ($F(2,153)=7.05$, $p<.001$). Sidak-corrected pairwise comparisons revealed a significant difference between controls and cocaine users with high craving ($p<.001$, $d=.69$). Introducing cumulative cocaine use as a further covariate did not change the results.

The ADHD-SR sum score was significantly correlated with mean %PPI across conditions in cocaine users as well ($r=.25$, $p<.02$). To test the influence of ADHD on PPI, cocaine users were divided into cocaine users with and without ADHD according to DSM-IV criteria and compared with controls **(Figure S5)**. A mixed effects ANCOVA (SOA*group) revealed a significant main effect for group

(F(2,153)=6.67, $p<.002$). Sidak-corrected pairwise comparisons revealed that cocaine users with ADHD showed a significantly increased PPI in comparison to controls ($p<.001$, $d=.76$). Interestingly, in cocaine users with ADHD PPI enhancement was most pronounced in the SOA 30 and 240 conditions. Cocaine use parameters did not differ between cocaine users with and without ADHD (all $p>.47$), and ADHD-SR sum score was not correlated with cocaine use parameters. Introducing cumulative cocaine use as a covariate in the mixed effects ANCOVA (SOA*group) revealed similar results (group: F(2,152)=5.57, $p<.005$).

When comparing cocaine users stratified for an ADHD diagnosis (yes/no) and craving (high/low) with controls (**Figure 4**), a mixed effects ANCOVA (SOA*group) revealed a significant main effect for group (F(4,151)=4.74, $p=.001$). Controls differed significantly from cocaine users with ADHD and high craving ($p<.03$, $d=.78$) and cocaine users without ADHD and high craving ($p<.03$, $d=.66$). Cocaine users without ADHD and low craving displayed normal PPI levels. Cocaine use parameters again did not differ between cocaine user groups. Introducing cumulative cocaine use as a covariate did not reveal other results.

Discussion

The present study demonstrates that both, RCU and DCU, showed clearly increased PPI levels compared to stimulant-naïve controls. Hair toxicologies and comprehensive psychiatric diagnostics allowed the analysis of a relatively pure and well-described group of cocaine users with little psychiatric comorbidities and without polytoxic drug use. Correlation analyses suggest a positive association between amount and duration of cocaine use and PPI. No significant differences between groups were found with regard to startle reactivity or habituation measures. Furthermore, PPI was significantly augmented in cocaine users with negative urine samples, while users who tested positive for cocaine displayed almost normal PPI levels at least in 60 and 240ms SOA conditions. Finally, cocaine users with ADHD symptoms and high craving showed the strongest increase in PPI, while cocaine users without ADHD but high craving still showed significantly enhanced PPI.

In the sole previous study, Efferen et al.(31) reported a statistical trend towards increased PPI in DCU, which was confirmed in the present investigation. Presumably because of the small sample size (10 cocaine users, 9 controls) and the analysis of only three out of six blocks of stimulus trials the effect did not reach significance before (31). In contrast to results obtained in short-term cocaine-withdrawn rats, showing no alterations in PPI (29,30), PPI seems to be sensitive to the lasting effects of cocaine use in humans and the species-specific results might be ascribed to differences in drug dose, route and duration of administration, abstinence period, and pharmacokinetics (55).

The PPI increase most likely reflects alterations in catecholamine neurotransmission:

1) As CSPP circuits regulating PPI and reward circuits, shown to be altered in cocaine users, overlap in the ventral striatum, changes of the striatal DA system are possible (10,11,22,23). This interpretation would be in line with previous studies reporting reduced striatal DA functioning in DCU by applying PET imaging (10-13). DA agonists decrease PPI in rodents (23,56) and humans (55,57). Furthermore, some DA-antagonistic antipsychotics increase PPI in rats (23,56,58,59) and humans (60,61), whereas others like haloperidol have been shown to have no effect or decrease PPI in humans (28,62,63). Thus, the role of striatal DA in the modulation the PPI might be more complex than a simple linear relationship between striatal DA concentration and PPI expression.

2) An increase of thalamic NE transporter density has been reported in DCU (16) and growing evidence suggests an involvement thalamocortical and ventral forebrain NE transmission in the regulation of PPI (25-28,64). Therefore, also dysregulation of NE transmission in CDU as well as RCU might contribute to the current results (17). Changes of the NE system would also be in line with the present and previous findings of slightly reduced startle magnitudes in cocaine users, because pharmacological as well as developmental lesions of the NE system can cause reductions of startle reactivity (65-67).

The finding that PPI was increased exclusively in cocaine users with negative urine samples further support the view that reduced catecholamine levels are responsible for this effect. Users with positive urine samples have used cocaine recently, which is supposed to enhance DA and NE levels and reduce PPI (29,57,68). Furthermore, increased PPI was significantly correlated with amount and duration of cocaine use, which suggests that the alterations in PPI might be substance-induced. PPI seems to be more sensitive to cumulative use compared to recent drug use patterns, as it did not correlate with weekly consumption or hair toxicologies, suggesting that rather long-term changes in catecholamine systems are associated with enhanced PPI. However, although correlations between PPI, cocaine use, and craving may indicate drug withdrawal-induced PPI augmentation, it cannot be ruled out that alterations in PPI and neurotransmitter signaling precede cocaine use and possibly represent a vulnerability to develop cocaine addiction.

Replicating earlier studies, PPI was reduced in women in the luteal phase of the ovarian cycle (51,52). This was explained by an increase in estrogen during the luteal phase, which results in elevated striatal DA release (69). However, as the distribution of women in the luteal and follicular phase did not differ between groups and menstrual cycle combined with sex was included as a covariate, it is unlikely that PPI differences between cocaine users and controls can be attributed to sex or hormonal changes.

In line with previous studies in humans (31) and rodents (30), startle reactivity and habituation were slightly reduced in DCU, but the differences did not reach significance. Though, previous results on startle reactivity have been somewhat inconsistent so far. Whereas Efferen et al.(31) reported

decreased startle magnitudes in early abstinent cocaine users, Corcoran et al.(32) referred reduced startle reactivity not until 40 days of abstinence. In our study, duration of self-reported cocaine abstinence was not correlated with startle reactivity (p=.80). Though, further studies are needed to disclose the influence of abstinence duration on startle reactivity and PPI in cocaine users.

Additionally, we examined the influence of craving and ADHD on PPI, as craving has been associated with dysregulated striatal DA and NE levels (12,17,18,36). Furthermore, dysfunction of NE and DA neurotransmission have been proposed to underlie the pathophysiology in ADHD (34,35,70). ADHD symptomatology and craving both increased PPI in cocaine users compared to controls, even if amount and duration of cocaine use were controlled. Low craving scores were associated with widely normal PPI, independent of ADHD diagnosis. Previous studies reported no significant effect of ADHD on PPI in adults (71,72). However, two studies reported slight but non-significant increases of PPI in untreated (n=13) or treatment-withdrawn (n=22) adults with ADHD (72,73), while stimulant-treated ADHD patients (n=10) displayed somewhat lower PPI levels (72). Perhaps, these studies were underpowered and therefore did not reveal an increase of PPI, even though the study of Feifel et al.(71) did not find PPI differences between unmedicated adult ADHD patients (n=20) and controls (n=17). Maybe the PPI-increasing influence of ADHD symptoms shown here only arises from an interaction of cocaine use and underlying ADHD pathophysiology. Taken together, our results might explain why ADHD patients seem to be more vulnerable for addiction than healthy subjects (74) because our results indicate that cocaine using ADHD individuals experience stronger craving symptoms (reflected by maximum PPI increase), possibly due to greater changes catecholamine systems. Additionally, given that we studied only unmedicated cocaine users, cocaine might also be utilized as a self-medication in our users showing ADHD symptoms (74). The need for self-medication might reflect a dysfunctional catecholamine system, which in turn could be more vulnerable for neurochemical plasticity induced by cocaine.

As cocaine users tested in this study showed minimal to moderate co-use of MDMA and cannabis, their influence on PPI was further analyzed. In previous studies, cannabis use did not show significant

effects on PPI at least passive attention paradigms just as used here (40,75,76). However, controls with positive cannabis urine toxicology showed a slight even though non-significant increase of PPI, which is in line with several animal studies observing that acute administration of cannabinoid-1 receptor agonists increase PPI (77,78). However, controls and cocaine users with negative cannabis urine samples still significantly differed, indicating that our result can not be explained by cannabis co-use (**Figure 2**). Moreover, we have previously shown that MDMA users also display elevated PPI levels (40) but here we did not find an additional increase of PPI in cocaine users with a limited co-use of MDMA. This difference may be explained by either a ceiling effect or by the exclusion of cocaine users with a regular or high use of MDMA in the present study. Given that also MDMA-naïve cocaine users displayed enhanced PPI levels (**Figure S7**) our results can not be attributed to the MDMA co-use of some users.

The study has some limitations: First, we have no objective measure of the duration of abstinence beyond urine toxicologies but have to rely on self-reports. Thus, we were unable to investigate the true effect of abstinence duration on startle parameters. However, it would have been nearly impossible to control for abstinence in our ambulant and voluntary study setting. Second, although this is one of the first investigations employing hair toxicologies in an electrophysiological study with cocaine users we can only rely on self-reports for illegal drug use prior to 3-6 months (depending on hair length), which is nevertheless an inevitable constraint in all studies with illegal drug users (79).

In sum, RCU and DCU showed an increase of PPI that was correlated with duration and amount of cocaine use, as well as the strength of cocaine craving. These data suggest that already recreational use of cocaine is associated with altered catecholamine signaling, which is in line with our previous finding of altered blue-yellow color vision in RCU (19). Moreover, the elevation of PPI was most pronounced in subjects with clinically relevant ADHD symptoms and high craving. PPI might therefore provide a non-invasive, simple, and cheap measure to objectively capture acute stimulant craving symptoms. Finally, our data imply that ADHD might be a critical risk factor for cocaine-induced changes of catecholamine systems.

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References

1. Nutt D, King LA, Saulsbury W, Blakemore C (2007): Development of a rational scale to assess the harm of drugs of potential misuse. *Lancet* 369:1047-1053.
2. European Monitoring Centre for Drugs and Drug Addiction (2011): Annual report 2011: the state of the drugs problem in Europe. In: Addiction EMCfDaD editor. Luxembourg: Publications Office of the European Union, p 105.
3. United Nations Office on Drugs and Crime (2011): World drug report 2011. Vienna, Austria: United Nations Office on Drugs and Crime.
4. Rothman RB, Baumann MH, Dersch CM, Romero DV, Rice KC, Carroll FI, et al (2001): Amphetamine-type central nervous system stimulants release norepinephrine more potently than they release dopamine and serotonin. *Synapse* 39:32-41.
5. Bolla K, Ernst M, Kiehl K, Mouratidis M, Eldreth D, Contoreggi C, et al (2004): Prefrontal cortical dysfunction in abstinent cocaine abusers. *J Neuropsychiatry Clin Neurosci* 16:456-464.
6. Volkow ND, Hitzemann R, Wang GJ, Fowler JS, Wolf AP, Dewey SL, et al (1992): Long-term frontal brain metabolic changes in cocaine abusers. *Synapse* 11:184-190.
7. Makris N, Gasic GP, Kennedy DN, Hodge SM, Kaiser JR, Lee MJ, et al (2008): Cortical thickness abnormalities in cocaine addiction--a reflection of both drug use and a pre-existing disposition to drug abuse? *Neuron* 60:174-188.
8. Ersche KD, Barnes A, Jones PS, Morein-Zamir S, Robbins TW, Bullmore ET (2011): Abnormal structure of frontostriatal brain systems is associated with aspects of impulsivity and compulsivity in cocaine dependence. *Brain* 134:2013-2024.
9. Kuhar MJ, Pilotte NS (1996): Neurochemical changes in cocaine withdrawal. *Trends Pharmacol Sci* 17:260-264.
10. Martinez D, Broft A, Foltin RW, Slifstein M, Hwang DR, Huang Y, et al (2004): Cocaine dependence and D2 receptor availability in the functional subdivisions of the striatum: relationship with cocaine-seeking behavior. *Neuropsychopharmacology* 29:1190-1202.
11. Volkow ND, Fowler JS, Wang GJ, Hitzemann R, Logan J, Schlyer DJ, et al (1993): Decreased dopamine D2 receptor availability is associated with reduced frontal metabolism in cocaine abusers. *Synapse* 14:169-177.
12. Volkow ND, Wang GJ, Fowler JS, Logan J, Gatley SJ, Hitzemann R, et al (1997): Decreased striatal dopaminergic responsiveness in detoxified cocaine-dependent subjects. *Nature* 386:830-833.
13. Martinez D, Narendran R, Foltin RW, Slifstein M, Hwang DR, Broft A, et al (2007): Amphetamine-induced dopamine release: markedly blunted in cocaine dependence and predictive of the choice to self-administer cocaine. *Am J Psychiatry* 164:622-629.
14. Jentsch JD, Taylor JR (1999): Impulsivity resulting from frontostriatal dysfunction in drug abuse: implications for the control of behavior by reward-related stimuli. *Psychopharmacology* 146:373-390.
15. Volkow ND, Wang GJ, Fowler JS (1997): Imaging studies of cocaine in the human brain and studies of the cocaine addict. *Annals of the New York Academy of Sciences* 820:41-54; discussion 54-45.
16. Ding YS, Singhal T, Planeta-Wilson B, Gallezot JD, Nabulsi N, Labaree D, et al (2010): PET imaging of the effects of age and cocaine on the norepinephrine transporter in the human brain using (S,S)-[(11)C]O-methylreboxetine and HRRT. *Synapse* 64:30-38.
17. Sofuoglu M, Sewell RA (2009): Norepinephrine and stimulant addiction. *Addict Biol* 14:119-129.
18. Erb S (2010): Evaluation of the relationship between anxiety during withdrawal and stress-induced reinstatement of cocaine seeking. *Prog Neuropsychopharmacol Biol Psychiatry* 34:798-807.
19. Hulka LM, Wagner M, Preller KH, Jenni D, Quednow BB (2012): Blue-yellow colour vision impairment and cognitive deficits in occasional and dependent stimulant users. *Int J Neuropsychopharmacol*:In press.
20. Graham FK (1975): The more or less startling effects of weak prestimulation. *Psychophysiology* 12:238-248.

21. Braff DL, Grillon C, Geyer MA (1992): Gating and habituation of the startle reflex in schizophrenic patients. *Arch Gen Psychiatry* 49:206-215.
22. Swerdlow NR, Braff DL, Geyer MA (1999): Cross-species studies of sensorimotor gating of the startle reflex. *Annals of the New York Academy of Sciences* 877:202-216.
23. Zhang J, Forkstam C, Engel JA, Svensson L (2000): Role of dopamine in prepulse inhibition of acoustic startle. *Psychopharmacology* 149:181-188.
24. Braff DL, Geyer MA, Swerdlow NR (2001): Human studies of prepulse inhibition of startle: normal subjects, patient groups, and pharmacological studies. *Psychopharmacology* 156:234-258.
25. Alsene KM, Bakshi VP (2011): Pharmacological stimulation of locus coeruleus reveals a new antipsychotic-responsive pathway for deficient sensorimotor gating. *Neuropsychopharmacology* 36:1656-1667.
26. Alsene KM, Rajbhandari AK, Ramaker MJ, Bakshi VP (2011): Discrete forebrain neuronal networks supporting noradrenergic regulation of sensorimotor gating. *Neuropsychopharmacology* 36:1003-1014.
27. Oranje B, Glenthoj BY (2012): Clonidine Normalizes Sensorimotor Gating Deficits in Patients With Schizophrenia on Stable Medication. *Schizophr Bull*.
28. Oranje B, Kahn RS, Kemner C, Verbaten MN (2004): Modulating sensorimotor gating in healthy volunteers: the effects of desipramine and haloperidol. *Psychiatry Res* 127:195-205.
29. Martinez ZA, Ellison GD, Geyer MA, Swerdlow NR (1999): Effects of sustained cocaine exposure on sensorimotor gating of startle in rats. *Psychopharmacology* 142:253-260.
30. Adams JU, Efferen TR, Duncan EJ, Rotrosen J (2001): Prepulse inhibition of the acoustic startle response in cocaine-withdrawn rats. *Pharmacol Biochem Behav* 68:753-759.
31. Efferen TR, Duncan EJ, Szilagyi S, Chakravorty S, Adams JU, Gonzenbach S, et al (2000): Diminished acoustic startle in chronic cocaine users. *Neuropsychopharmacology* 22:89-96.
32. Corcoran S, Norrholm SD, Cuthbert B, Sternberg M, Hollis J, Duncan E (2011): Acoustic startle reduction in cocaine dependence persists for 1 year of abstinence. *Psychopharmacology* 215:93-103.
33. Perez de Los Cobos J, Sinol N, Puerta C, Cantillano V, Lopez Zurita C, Trujols J (2011): Features and prevalence of patients with probable adult attention deficit hyperactivity disorder who request treatment for cocaine use disorders. *Psychiatry Res* 185:205-210.
34. Del Campo N, Chamberlain SR, Sahakian BJ, Robbins TW (2011): The roles of dopamine and noradrenaline in the pathophysiology and treatment of attention-deficit/hyperactivity disorder. *Biol Psychiatry* 69:e145-157.
35. Volkow ND, Wang GJ, Kollins SH, Wigal TL, Newcorn JH, Telang F, et al (2009): Evaluating dopamine reward pathway in ADHD: clinical implications. *Jama* 302:1084-1091.
36. Diana M (2011): The dopamine hypothesis of drug addiction and its potential therapeutic value. *Front Psychiatry* 2:64.
37. Prinzleve M, Haasen C, Zurhold H, Matali JL, Bruguera E, Gerevich J, et al (2004): Cocaine use in Europe - a multi-centre study: patterns of use in different groups. *Eur Addict Res* 10:147-155.
38. Degenhardt L, Hall W (2012): Extent of illicit drug use and dependence, and their contribution to the global burden of disease. *Lancet* 379:55-70.
39. American Psychiatric Association (1994): *Diagnostic and statistical manual of mental disorders: DSM-IV*, 4th ed. Washington, DC: American Psychiatric Association (APA).
40. Quednow BB, Kuhn K-U, Hoenig K, Maier W, Wagner M (2004): Prepulse inhibition and habituation of acoustic startle response in male MDMA ("Ecstasy") users, cannabis users, and healthy controls. *Neuropsychopharmacology* 29:982-990.
41. Tiffany ST, Singleton E, Haertzen CA, Henningfield JE (1993): The development of a cocaine craving questionnaire. *Drug Alcohol Depend* 34:19-28.
42. Heatherton TF, Kozlowski LT, Frecker RC, Fagerstrom KO (1991): The Fagerstrom Test for Nicotine Dependence: a revision of the Fagerstrom Tolerance Questionnaire. *Br J Addict* 86:1119-1127.
43. Lehrl S (1999): *Mehrfachwahl-Wortschatz-Intelligenztest (MWT-B)*, 4 ed. Göttingen: Hogrefe.
44. Beck AT, Ward CH, Mendelson M, Mock J, Erbaugh J (1961): An inventory for measuring depression. *Arch Gen Psychiatry* 4:561-571.

45. Rosler M, Retz W, Retz-Junginger P, Thome J, Supprian T, Nissen T, et al (2004): Instrumente zur Diagnostik der Aufmerksamkeitsdefizit-/Hyperaktivitätsstörung (ADHS) im Erwachsenenalter: Selbstbeurteilungsskala (ADHS-SB) und Diagnosecheckliste (ADHS-DC). *Nervenarzt* 75:888-895.
46. Csomor PA, Yee BK, Vollenweider FX, Feldon J, Nicolet T, Quednow BB (2008): On the influence of baseline startle reactivity on the indexation of prepulse inhibition. *Behav Neurosci* 122:885-900.
47. Quednow BB, Kuhn KU, Beckmann K, Westheide J, Maier W, Wagner M (2006): Attenuation of the prepulse inhibition of the acoustic startle response within and between sessions. *Biol Psychol* 71:256-263.
48. Duncan E, Madonick S, Chakravorty S, Parwani A, Szilagyi S, Efferen T, et al (2001): Effects of smoking on acoustic startle and prepulse inhibition in humans. *Psychopharmacology* 156:266-272.
49. Kumari V, Gray JA (1999): Smoking withdrawal, nicotine dependence and prepulse inhibition of the acoustic startle reflex. *Psychopharmacology* 141:11-15.
50. Ludewig K, Ludewig S, Seitz A, Obrist M, Geyer MA, Vollenweider FX (2003): The acoustic startle reflex and its modulation: effects of age and gender in humans. *Biol Psychol* 63:311-323.
51. Swerdlow NR, Hartman PL, Auerbach PP (1997): Changes in sensorimotor inhibition across the menstrual cycle: implications for neuropsychiatric disorders. *Biol Psychiatry* 41:452-460.
52. Jovanovic T, Szilagyi S, Chakravorty S, Fiallos AM, Lewison BJ, Parwani A, et al (2004): Menstrual cycle phase effects on prepulse inhibition of acoustic startle. *Psychophysiology* 41:401-406.
53. Quednow BB, Wagner M, Westheide J, Beckmann K, Bliesener N, Maier W, et al (2006): Sensorimotor gating and habituation of the startle response in schizophrenic patients randomly treated with amisulpride or olanzapine. *Biol Psychiatry* 59:536-545.
54. Blumenthal T (1999): Short lead interval startle modification. In: Dawson M, Schell A, Böhmelt A editors. *Startle Modification Implications for Neuroscience, Cognitive Sciences, and Clinical Science*. Cambridge UK: Cambridge University Press, pp 51–71.
55. Swerdlow NR, Stephany N, Wasserman LC, Talledo J, Shoemaker J, Auerbach PP (2003): Amphetamine Effects on Prepulse Inhibition Across-Species: Replication and Parametric Extension. *Neuropsychopharmacology* 28:640-650.
56. Johansson C, Jackson DM, Zhang J, Svensson L (1995): Prepulse inhibition of acoustic startle, a measure of sensorimotor gating: effects of antipsychotics and other agents in rats. *Pharmacol Biochem Behav* 52:649-654.
57. Hutchison KE, Swift R (1999): Effect of d-amphetamine on prepulse inhibition of the startle reflex in humans. *Psychopharmacology* 143:394-400.
58. Swerdlow NR, Geyer MA (1993): Clozapine and haloperidol in an animal model of sensorimotor gating deficits in schizophrenia. *Pharmacol Biochem Behav* 44:741-744.
59. Depoortere R, Perrault G, Sanger DJ (1997): Some, but not all, antipsychotic drugs potentiate a low level of prepulse inhibition shown by rats of the Wistar strain. *Behav Pharmacol* 8:364-372.
60. Vollenweider FX, Barro M, Csomor PA, Feldon J (2006): Clozapine enhances prepulse inhibition in healthy humans with low but not with high prepulse inhibition levels. *Biol Psychiatry* 60:597-603.
61. Holstein DH, Csomor PA, Geyer MA, Huber T, Brugger N, Studerus E, et al (2011): The effects of sertindole on sensory gating, sensorimotor gating, and cognition in healthy volunteers. *J Psychopharmacol* 25:1600-1613.
62. Kumari V, Mulligan OF, Cotter PA, Poon L, Toone BK, Checkley SA, et al (1998): Effects of single oral administrations of haloperidol and d-amphetamine on prepulse inhibition of the acoustic startle reflex in healthy male volunteers. *Behav Pharmacol* 9:567-576.
63. Abduljawad KA, Langley RW, Bradshaw CM, Szabadi E (1998): Effects of bromocriptine and haloperidol on prepulse inhibition of the acoustic startle response in man. *J Psychopharmacol* 12:239-245.

64. Quednow BB, Kuhn KU, Stelzenmuelle R, Hoenig K, Maier W, Wagner M (2004): Effects of serotonergic and noradrenergic antidepressants on auditory startle response in patients with major depression. *Psychopharmacology (Berl)* 175:399-406.
65. Adams LM, Geyer MA (1981): Effects of 6-hydroxydopamine lesions of locus coeruleus on startle in rats. *Psychopharmacology (Berl)* 73:394-398.
66. Von Coelln R, Thomas B, Savitt JM, Lim KL, Sasaki M, Hess EJ, et al (2004): Loss of locus coeruleus neurons and reduced startle in parkin null mice. *Proc Natl Acad Sci U S A* 101:10744-10749.
67. Swerdlow N, Bongiovanni M, Tochen L, Shoemaker J (2006): Separable noradrenergic and dopaminergic regulation of prepulse inhibition in rats: implications for predictive validity and Tourette Syndrome. *Psychopharmacology* 186:246-254.
68. Ritz MC, Lamb RJ, Goldberg SR, Kuhar MJ (1987): Cocaine receptors on dopamine transporters are related to self-administration of cocaine. *Science* 237:1219-1223.
69. Becker JB (1999): Gender differences in dopaminergic function in striatum and nucleus accumbens. *Pharmacol Biochem Behav* 64:803-812.
70. Howells FM, Stein DJ, Russell VA (2012): Synergistic tonic and phasic activity of the locus coeruleus norepinephrine (LC-NE) arousal system is required for optimal attentional performance. *Metab Brain Dis* 27:267-274.
71. Feifel D, Minassian A, Perry W (2009): Prepulse inhibition of startle in adults with ADHD. *J Psychiatr Res* 43:484-489.
72. Hanlon MC, Karayanidis F, Schall U (2009): Intact sensorimotor gating in adult attention deficit hyperactivity disorder. *Int J Neuropsychopharmacol* 12:701-707.
73. Holstein DB, Vollenweider FX, Geyer MA, Csomor PA, Belser N, Eich D (2012): Sensory and sensorimotor gating in adult attention-deficit/hyperactivity disorder (ADHD). *Psychiatry Res* in press.
74. Gudjonsson GH, Sigurdsson JF, Sigfusdottir ID, Young S (2012): An epidemiological study of ADHD symptoms among young persons and the relationship with cigarette smoking, alcohol consumption and illicit drug use. *J Child Psychol Psychiatry* 53:304-312.
75. Kedzior KK, Martin-Iverson MT (2006): Chronic cannabis use is associated with attention-modulated reduction in prepulse inhibition of the startle reflex in healthy humans. *J Psychopharmacol* 20:471-484.
76. Kedzior KK, Martin-Iverson MT (2007): Attention-dependent reduction in prepulse inhibition of the startle reflex in cannabis users and schizophrenia patients--a pilot study. *Eur J Pharmacol* 560:176-182.
77. Stanley-Cary CC, Harris C, Martin-Iverson MT (2002): Differing effects of the cannabinoid agonist, CP 55,940, in an alcohol or Tween 80 solvent, on prepulse inhibition of the acoustic startle reflex in the rat. *Behav Pharmacol* 13:15-28.
78. Long LE, Chesworth R, Huang XF, McGregor IS, Arnold JC, Karl T (2010): A behavioural comparison of acute and chronic Delta9-tetrahydrocannabinol and cannabidiol in C57BL/6JArc mice. *Int J Neuropsychopharmacol* 13:861-876.
79. Curran HV (2000): Is MDMA ('Ecstasy') neurotoxic in humans? An overview of evidence and of methodological problems in research. *Neuropsychobiology* 42:34-41.

Figure legends

Fig. 1 – Mean percent prepulse inhibition (%PPI) in recreational (n=64) and dependent cocaine users (n=29), and healthy control participants (n=66). Recreational and dependent cocaine users show significantly increased PPI. Error bars refer to SEM. * indicates significant difference between groups ($p<0.05$).

Fig. 2: Mean %PPI in control participants with positive (n=12) and negative (n=54) urine samples for cannabis, and cocaine users with positive (n=34) and negative (n=59) urine samples for cannabis. Error bars refer to SEM. *indicates significant difference between groups (Sidak-post hoc test: $p<0.05$).

Figure 3: Mean %PPI across SOA conditions in non-smoking controls (controls, ns) (n=16) smoking controls (controls, s) (n=50), non-smoking cocaine users (cocaine users, ns) (n=19), and smoking cocaine users (cocaine users, s) (n=74). Error bars refer to SEM. *indicates significant difference between groups (Sidak-post hoc test: $p<0.05$).

Fig. 4 – Mean percent prepulse inhibition (%PPI) in cocaine users with ADHD and high craving (n=10), cocaine users with ADHD and low craving (n=12), cocaine users without ADHD and high craving (n=31), cocaine users with ADHD and low craving (n=40), and controls (n=66). High craving cocaine users with and without ADHD show significantly augmented PPI, while cocaine users without craving and ADHD display widely normal PPI levels. Error bars refer to SEM. *indicates significant difference between groups (Sidak post hoc test: $p<0.05$).

Table 1 Demographic data (means and standard deviation)

	Control group (n=66)	Recreational cocaine users (RCU, n=64)	Dependent cocaine users (DCU, n=29)	Value	df/df_{err}	p
Male/ female follicular/female luteal phase (n)	46/15/5	46/9/9	25/3/1	$\chi 2 = 5.94$ [b]	4	0.20
Age (years)	31.11 (9.40)	28.11 (6.24)	36.28 (11.45)*°	F = 8.80[c]	2/156	**<0.001**
Years of education	10.67 (1.87)	10.42 (1.91)	9.57 (1.24)*	F = 3.89[c]	2/156	**0.02**
Verbal IQ	106.09 (11.20)	102.44 (9.00)	102.10 (10.97)	F = 2.57[c]	2/156	0.08
Smoker/nonsmoker (n)	50/16	51/13	23/6	$\chi 2 = 0.33$[b]	2	0.85
FTND sum score (0-10)[a]	2.24 (2.11)	3.29 (2.10)*	4.87 (2.53)**°	F = 11.56[c]	2/122	**<0.001**
Craving for cocaine (0-70)	-	18.92 (9.01)	18.97 (10.55)	t = 0.02[d]	91	0.98
ADHD-SR sum score (0-22)	7.74 (4.75)	13.39 (9.11)**	15.50 (9.16)**	F = 13.88[c]	2/156	**<0.001**
BDI sum score (0-63)	4.42 (4.38)	7.70 (6.76)**	10.34 (7.58)**	F = 10.73[c]	2/156	**<0.001**
Hair color (n) (black/brown/blonde/dyed)	4/54/3/2	6/64/3/1	6/21/2/0	$\chi^2 = 5.94$[b]	6	0.43

Significant *p* values are shown in bold. Sex and smoking in frequency data. FTND, Fagerstrom Test of Nicotine Dependence; ADHD, ADHD self rating scale; BDI, Beck Depression Inventory.
[a]FTND measured in smokers only. [b]χ^2 test (all groups) for frequency data. [c]ANOVA (all groups). [d]Independent t-test (cocaine users only)
*indicates significant post-hoc test (Sidak) vs. control group: $p<0.05$
**indicates significant post-hoc test (Sidak) vs. control group: $p<0.01$
°indicates significant post-hoc test (Sidak) vs. recreational cocaine users group: $p<0.05$

Table 2 Pattern and amount of drug use: Results of the Psychotropic Drug Interview, urine toxicology, and hair samples

	Control group (n=66)	Recreational cocaine users (RCU, n=64)	Dependent cocaine users (DCU, n=29)	Value[a]	df/dferr	p
Cocaine						
times per week[b]	-	1.11 (1.08)	2.85 (2.66)	F = 20.14	1/91	**<0.001**
grams/week[b]	-	1.18 (1.46)	8.32 (16.15)	F = 12.48	1/91	**<0.001**
years of use	-	6.08 (3.93)	10.35 (6.91)	F = 14.30	1/91	**<0.001**
maximum dose (24h)	-	3.27 (2.37)	10.08 (8.49)	F = 35.51	1/91	**<0.001**
last consumption (days)	-	24.70 (35.99)	28.39 (38.19)	F = 0.19	1/91	0.66
cumulative dose (grams)	-	500.61 (734.45)	7211.70 (10503.26)	F = 26.19	1/91	**<0.001**
urine toxicology (pos/neg)	-	13/51	13/16	χ^2 = 5.73	1	**0.02**
hair sample (pg/mg)						
cocaine	-	2780.94 (4695.09)	19967.93 (33082.52)	F = 16.75	1/91	**<0.001**
benzoylecgonine	-	565.51 (932.77)	4313.62 (7531.84)	F = 15.53	1/91	**<0.001**
ethylcocaine	-	271.83 (312.28)	1879.31 (3721.96)	F = 11.91	1/91	**<0.001**
norcocaine	-	63.45 (101.19)	501.64 (739.20)	F = 21.87	1/91	**<0.001**
MDMA						
tablets/week[b]	-	0.09 (0.27)	0.42 (1.86)	F = 1.91	1/91	0.17
years of use	1.60 (11.31)	2.51 (3.81)	2.97 (5.35)	F = 0.37	2/156	0.69
last consumption (days)[c]	-	66.33 (83.34) (n=20)	77.20 (45.91) (n=8)	F = 0.12	1/26	0.73
cumulative dose (tablets)	0.61 (1.86)	37.70 (92.94)	139.10 (400.50)**°	F = 6.00	2/156	**<0.01**
hair sample (pg/mg)	2.74 (16.24)	627.24 (1660.04)**	240.86 (663.88)	F = 5.15	2/153	**<0.01**
Cannabis						
grams/week[b]	0.53 (1.47)	0.87 (2.01)	1.85 (4.84)	F = 2.63	2/156	0.08
years of use	5.68 (7.32)	7.56 (5.65)	10.35 (10.66)*	F = 4.02	2/156	**0.02**
last consumption (days)[c]	37.35 (52.23) (n=32)	20.67 (30.84) (n=43)	24.94 (30.72) (n=17)	F = 1.66	2/89	0.20
cumulative dose (grams)	426.73 (903.46)	1082.74 (1780.72)	4491.36 (7478.60)**°°	F = 14.84	2/156	**<0.001**
urine toxicology (pos/neg)	12/54	23/40	11/18	χ^2 = 6.58	2	**0.04**

Table 2 Pattern and amount of drug use: Results of the Psychotropic Drug Interview, urine toxicology, and hair samples (continued)

	Control group (n=66)	Recreational cocaine users (RCU, n=64)	Dependent cocaine users (DCU, n=29)	Value[a]	df/dferr	p
Amphetamine						
grams/week[b]	-	0.08 (0.21)**	0.01 (0.04)	F = 2.58	1/92	0.11
years of use	0.01 (0.06)	1.64 (2.99)**	1.48 (3.23)*	F = 8.87	2/156	**<0.001**
last consumption (days)[c]	-	63.10 (52.08) (n=24)	93.06 (74.12) (n=5)	F = 1.19	1/27	0.29
cumulative dose (grams)	0.17 (1.44)	22.58 (59.01)*	16.70 (61.01)	F = 4.10	2/156	**0.02**
hair sample (pg/mg)	0.9 (7.56)	75.47 (259.52)	44.31 (158.97)	F = 2.73	2/153	0.07
GHB						
cumulative dose (pipettes)	-	1.79 (9.77)	1.14 (2.89)	F = 0.12	1/91	0.73
Hallucinogenes						
cumulative dose (times)	1.80 (7.13)	6.78 (15.14)	8.07 (15.37)	F = 3.69	2/156	**0.03**
Alcohol						
grams/week[b]	118.83 (126.58)	174.96 (118.65)	196.09 (286.64)	F = 2.97	2/156	0.05
years of use	13.83 (9.61)	10.84 (5.08)	14.73 (9.80)	F = 3.22	2/156	**0.04**
Nicotine						
cigarettes per day (CPD)[b]	9.03 (9.49)	11.96 (8.33)	15.60 (14.20)*	F = 4.43	2/156	**0.01**
years of use	9.52 (9.68)	9.53 (6.34)	15.78 (11.33)**°°	F = 5.91	2/156	**<0.01**

Means and standard deviations in parenthesis. Significant p values are shown in bold. Consumption per week, duration of use, and cumulative dose are averaged within the total group.

[a]ANOVA or $\chi 2$ test for frequency data

[b]average use during the last six months

[c]Last consumption is averaged only for persons who used the drug in the last 6 months. In this case, sample size (n) is shown.

*indicates significant post-hoc test (Sidak) vs. control group: $p<0.05$

**indicates significant post-hoc test (Sidak) vs. control group: $p<0.01$

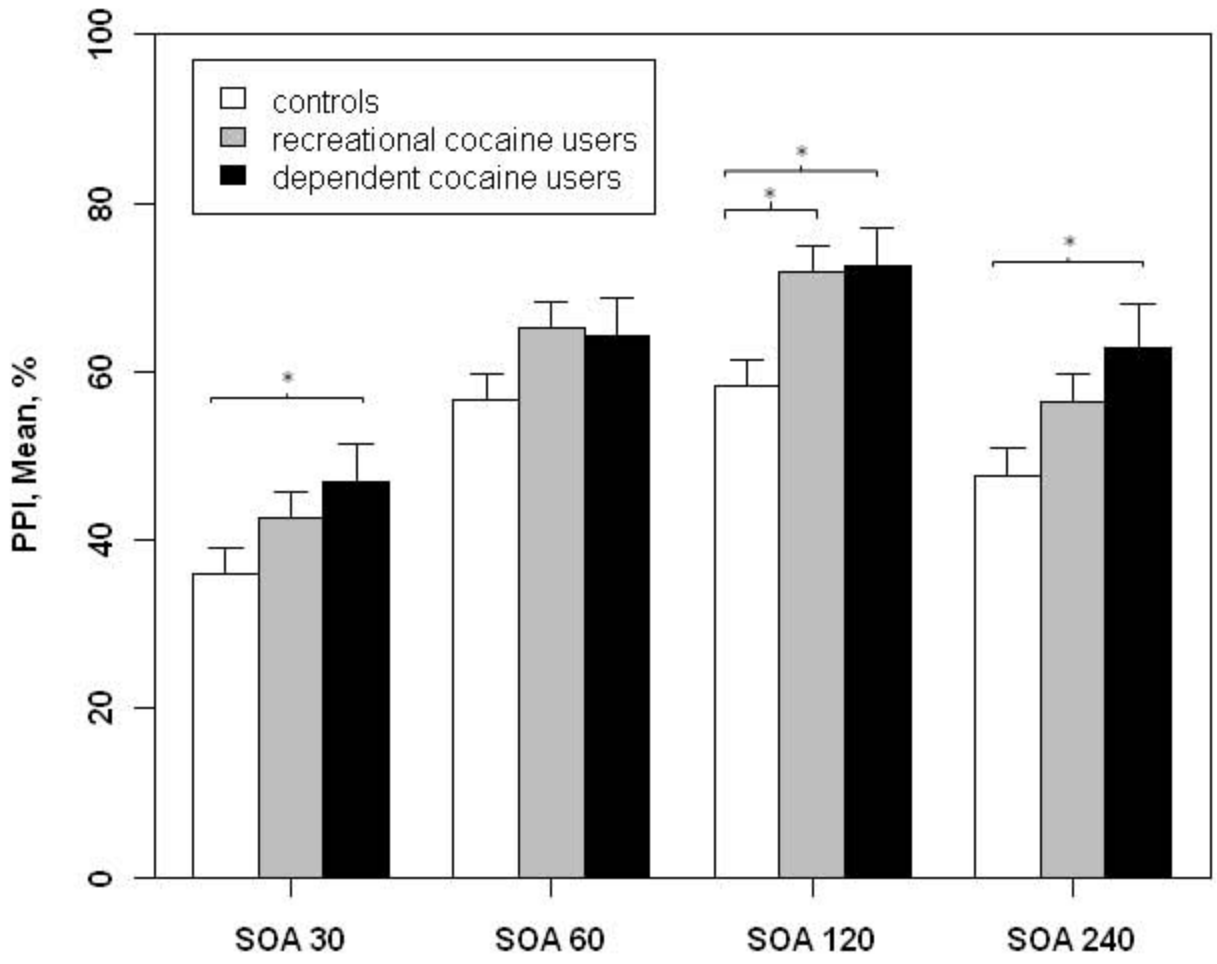
°indicates significant post-hoc test (Sidak) vs. recreational cocaine users group: $p<0.05$

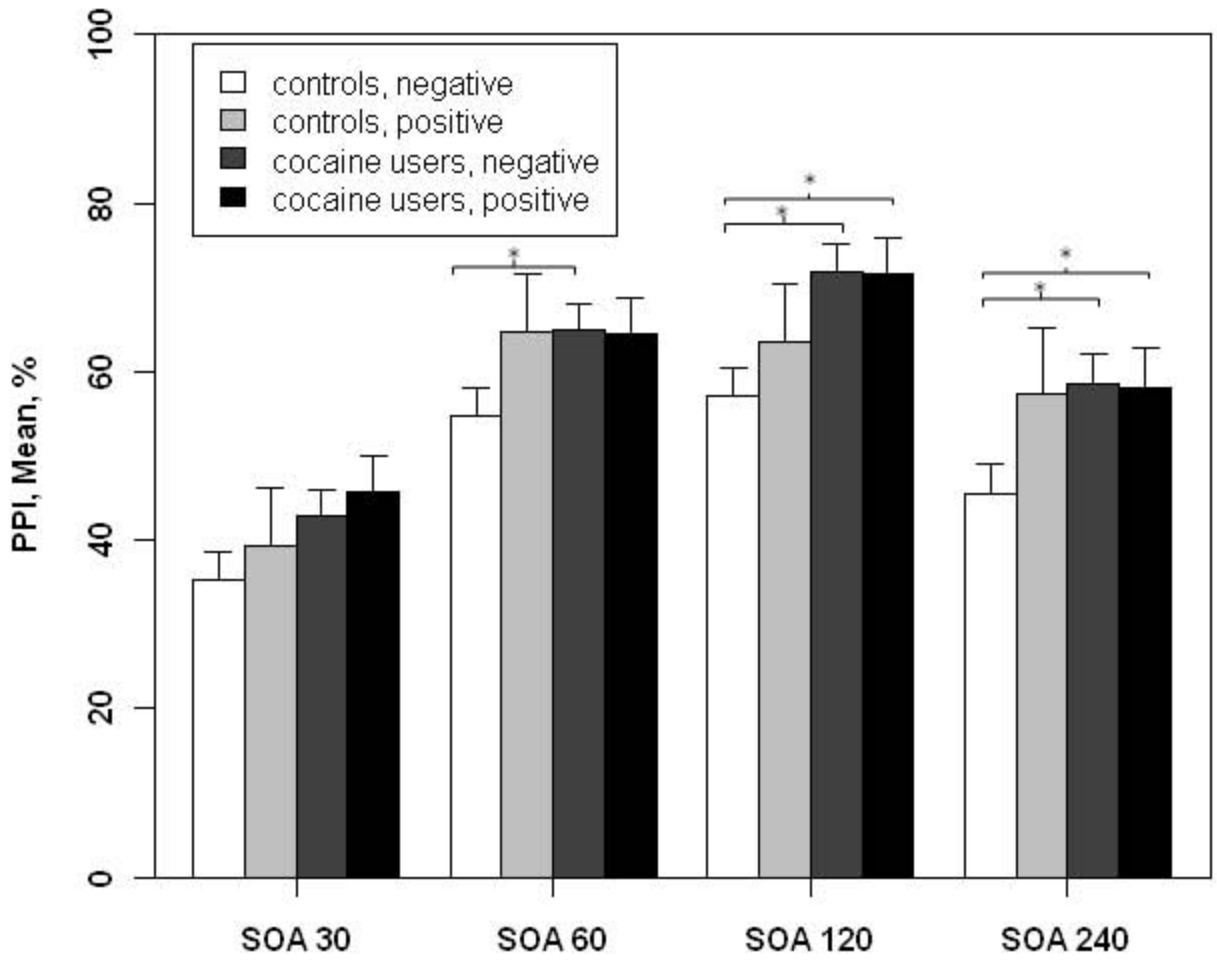
°° indicates significant post-hoc test (Sidak) vs. recreational cocaine users group: $p<0.01$

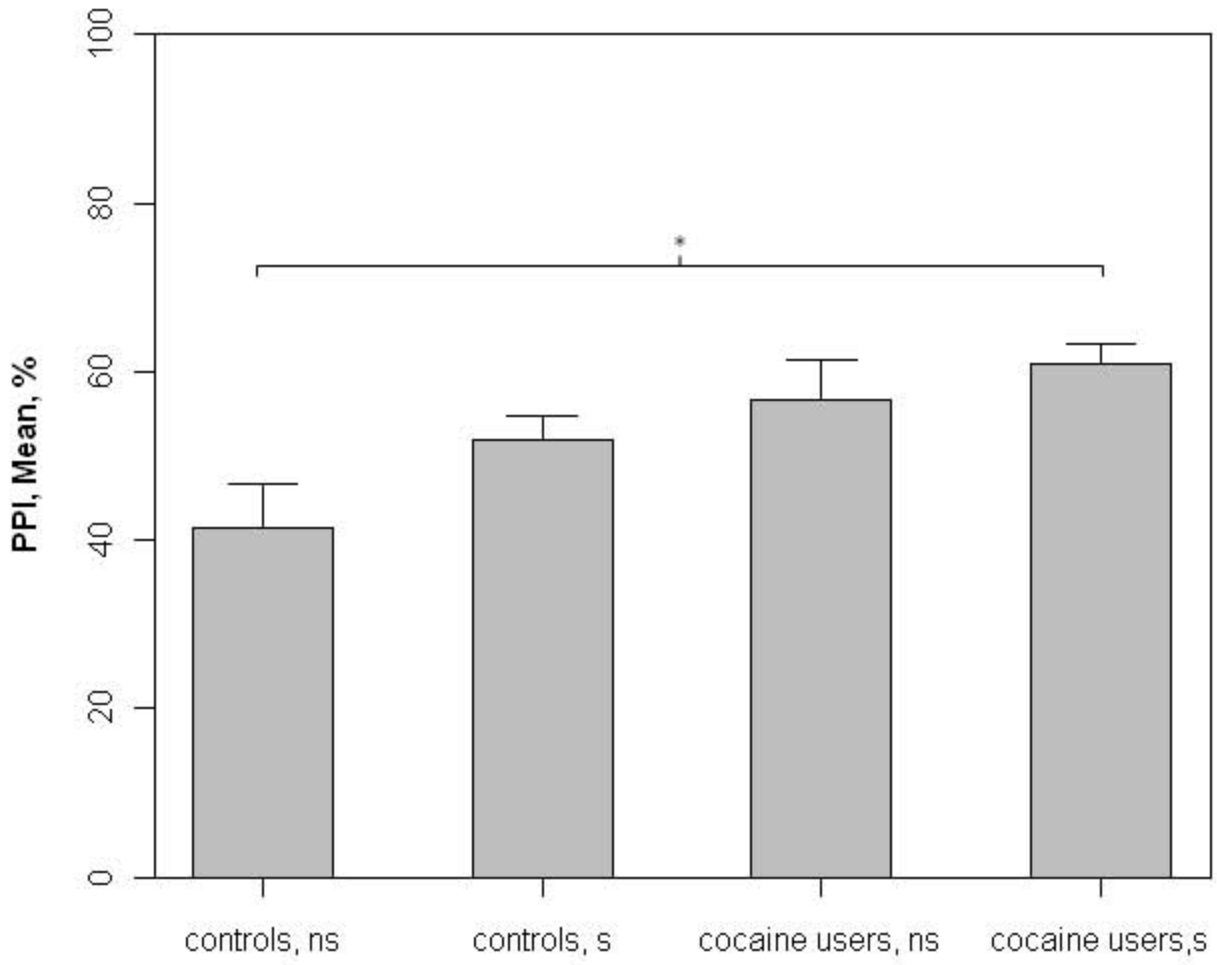
Table 3 Startle reactivity, mean %PPI and habituation

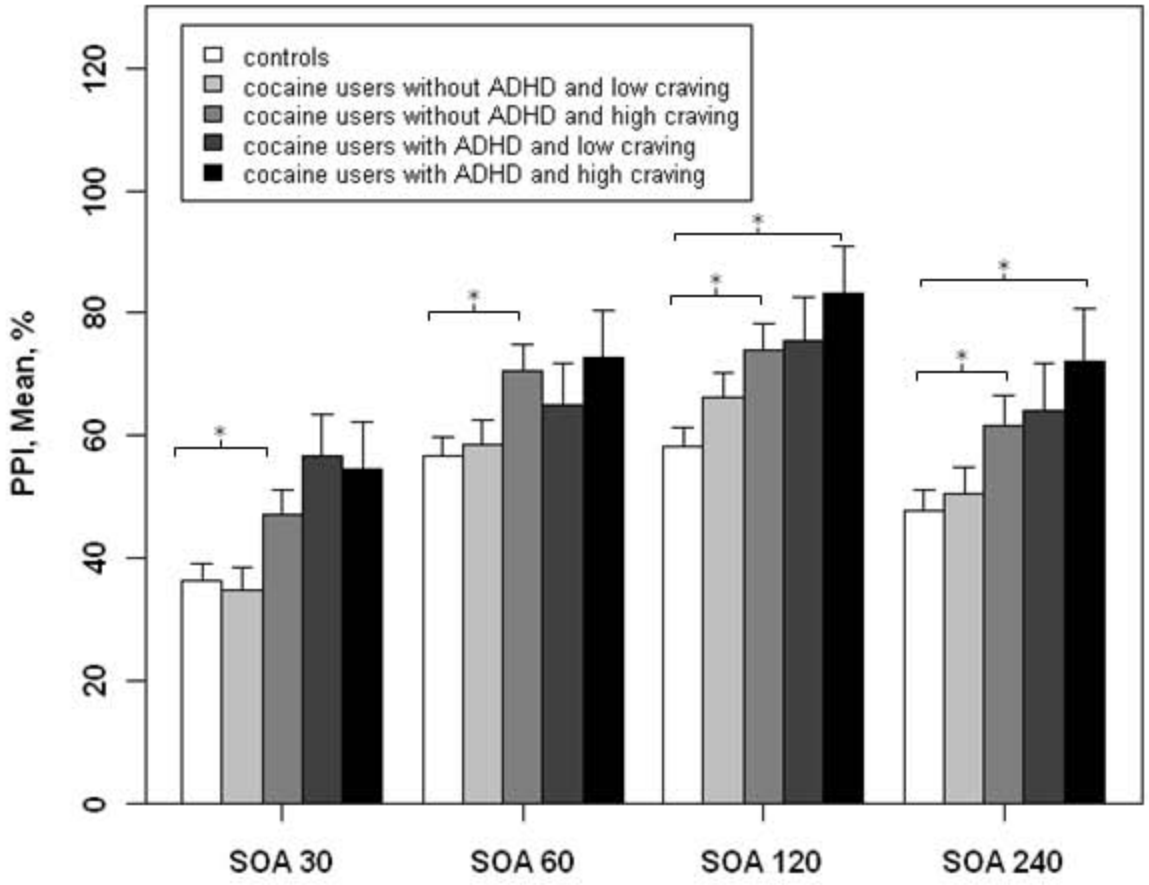
	Control group (n=66)	Recreational cocaine users (RCU, n=64)	Dependent cocaine users (DCU, n=29)	Value[a]	df/dferr	p
PA first block (μV)	121.52 (103.54)	120.72 (68.46)	91.19 (61.15)	F = 0.70	2/153	0.50
PA mean across blocks (μV)	82.00 (61.23)	76.74 (51.13)	58.57 (45.29)	F = 0.88	2/153	0.42
PPA mean (μV)	6.60 (6.72)	6.40 (5.39)	5.28 (3.12)	F = 0.31	2/153	0.74
Baseline (μV)	3.07 (1.39)	2.96 (1.30)	3.04 (1.34)	F = 0.63	2/153	0.53
%PPI mean across conditions	49.68 (23.02)	57.83 (20.88)*	64.15 (15.11)*	F = 4.58	2/153	**0.01**
Habituation						
%habituation block 2-3	20.05 (37.85)	23.17 (31.99)	12.23 (52.26)	F = 0.83	2/153	0.44
%habituation block 2-4	25.68 (41.41)	26.91 (47.11)	32.10 (36.62)	F = 0.32	2/153	0.72
coefficient *b*	-21.68 (28.64)	-21.34 (16.37)	-19.06 (14.48)	F = 0.12	2/153	0.89

Means and standard deviations in parenthesis. Significant p values are shown in bold.
PA, pulse alone trials; PPA, prepulse alone trial; PPI, prepulse inhibition
[a]ANCOVA including all groups (sex, age and smoking status as covariates)
*indicates significant post-hoc test (Sidak) vs. control group: $p<0.05$


controls
recreational cocaine users
dependent cocaine users
PPI, Mean, %
0
20
40
60
80
100
SOA 30
SOA 60
SOA 120
SOA 240
*
*
*
*


controls, negative
controls, positive
cocaine users, negative
cocaine users, positive
PPI, Mean, %
0
20
40
60
80
100
SOA 30
SOA 60
SOA 120
SOA 240
*
*
*
*
*
*


PPI, Mean, %
0
20
40
60
80
100
*
controls, ns
controls, s
cocaine users, ns
cocaine users,s


controls
cocaine users without ADHD and low craving
cocaine users without ADHD and high craving
cocaine users with ADHD and low craving
cocaine users with ADHD and high craving
PPI, Mean, %
0
20
40
60
80
100
120
SOA 30
SOA 60
SOA 120
SOA 240
*
*
*
*
*

Increased Sensorimotor Gating in Recreational and Dependent Cocaine Users is Modulated by Craving and Attention-Deficit/Hyperactivity Disorder Symptoms

Supplemental Information

Supplemental Methods

Recruitment and Selection Details

Participants were recruited in the Zurich area by means of advertisements in local newspapers, drug prevention and treatment centers, psychiatric hospitals, and internet platforms. Eight-hundred-four potential participants completed an initial telephone screening, whereof 240 subjects participated in the study. Forty-six participants had to be excluded afterwards because of hair analyses revealing illegal drug use not declared in the interviews (e.g., opioids, excessive 3,4-methylenedioxy-*N*-methylamphetamine (MDMA) use or polydrug use) or lack of cocaine use. Furthermore, the startle data of 19 participants (11 controls, 8 cocaine users) could not be analyzed because of technical problems/equipment malfunction during the test session. Further 16 participants were excluded due to matching reasons (age, IQ, education, and smoking) between groups (9 controls, 1 cocaine user), startle non-responding (2 controls), and hearing problems (4 cocaine users). Therefore, 159 datasets were included in the analysis. Hair samples were provided by 156 subjects, as hair analysis was not possible by reason of an insufficient amount of hair for three control subjects. Women provided information on the days since their last menstruation. Women in the first 14 days of the cycle were considered in the follicular phase, in the last 14 days in the luteal phase.

Urine and Hair Toxicologies

Urine toxicology analyses comprised the compounds/substances: tetrahydrocannabinol, cocaine, amphetamines, benzodiazepines, opioids, and methadone and were assessed by a semi-quantitative enzyme multiplied immunoassay method using a Dimension RXL Max (Siemens, Erlangen, Germany).

To characterize drug use over the last six months objectively, hair samples were collected and analyzed with liquid chromatography-tandem mass spectrometry (LC-MS/MS). If participants' hair

was long enough, one sample of six cm hair (from the scalp) was taken and subsequently divided into two subsamples of three cm length. The following compounds were assessed: cocaine, benzoylecgonine, ethylcocaine, norcocaine, amphetamine, methamphetamine, MDMA, 3,4-methylenedioxy-*N*-ethylamphetamine (MDEA), 3,4-methylenedioxyamphetamine (MDA), morphine, codeine, methadone EDDP (2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine; primary methadone metabolite), tramadol, and methylphenidate.

For our routine protocol for drugs of abuse analysis, a three step washing procedure with water (2 minutes shaking, 15 ml), acetone (2 min., 10 ml) and finally hexane (2 min., 10 ml) of hair was performed. Then the hair samples were dried at ambient temperatures, cut into small snippets and extracted in two steps, first with methanol (5 ml, 16 hours, ultrasonication) and a second step with 3 ml MeOH acidified with 50 µL hydrochloric acid 33% (3 hours, ultrasonication). The extracts were dried and the residue reconstituted with 50 µL MeOH and 500 µL 0.2 mM ammonium formate (analytical grade) in water. As internal standards deuterated standards of the following compounds were used, added as mixture of the following compounds: cocaine-d3, benzoylecgonine-d3, ethylcocaine-d3, morphine-d3, MAM-d3, codeine-d3, dihydrocodeine-d3, amphetamine-d6, methamphetamine-d9, MDMA-d5, MDEA-d6, MDA-d5, methadone-d9, EDDP-d3, methylphenidate-d9, tramadol-d3, oxycodone-d3, and ephedrine-d3. All deuterated standards were from ReseaChem (Burgdorf, Switzerland), the solvents for washing and extraction were of analysis grade and obtained from Merck (Darmstadt, Germany); LC-solvents were of high-performance LC grade and were obtained from Sigma Aldrich (Buchs, Switzerland).

The LC-MS/MS apparatus was an ABSciex QTrap 3200 (Analyst software Version 1.5, Turbo V ion source operated in the ESI mode, gas 1, nitrogen (50 psi); gas 2, nitrogen (60 psi); ion spray voltage, 3500V; ion source temperature, 450°C; curtain gas, nitrogen (20 psi) collision gas, medium), with a Shimadzu Prominence LC-system (Shimadzu CBM 20 A controller, two Shimadzu LC 20 AD pumps including a degasser, a Shimadzu SIL 20 AC autosampler and a Shimadzu CTO 20 AC column oven, Shimadzu, Duisburg, Germany). Gradient elution was performed on a separation column (Synergi 4µ POLAR-RP 80A, 150x2.0 with a POLAR-RP 4x2.0 Security Guard Cartridge, (Phenomenex, Aschaffenburg, Germany). The mobile phase consisted of 1 mM ammonium formate

buffer adjusted to pH 3.5 with formic acid (eluent A) and acetonitrile containing 1 mM ammonium formate and 1 mM formic acid (eluent B). The analysis was performed in multiple reaction monitoring mode with two transitions per analyte and one transition for each deuterated internal standard, respectively.

Startle Response Measurement

Electromyographic (EMG) recording was performed in a sound-attenuated room. All participants underwent a short hearing test before the test session to ensure hearing within normal limits. Participants were informed that they would hear broadband noise and bursts over the headphones. They were asked to sit comfortably, stay awake, and keep their eyes open. The eye-blink component of the acoustic startle response was measured by an EMG startle system (EMG-SR-Lab; San Diego Instruments, Inc., San Diego, CA). EMG activity was measured from the right *orbicularis oculi* muscle using two silver/silver chloride electrodes. A reference electrode was placed on the glabella. All electrode resistances were less than 10 kΩ. The system recorded continuously over the whole session with a sampling rate of 4096 Hz. Acoustic startle stimuli were presented binaurally using headphones (TDH-39-P; Maico). The test session started with a 2-min acclimation period of 70 dB background broadband noise that was continued throughout the session. Startle stimuli comprised of noise bursts at an 115 dB sound pressure level with duration of 40 ms, separated by variable intertrial intervals (range: 9-14 seconds, mean: 12 seconds). After an initial pulse-alone trial (PA) the session included a total of 64 trials (56 active and 8 no-stimulation trials) and lasted 13 minutes. Thirty-two pulse trials were preceded by a 20 ms prepulse with an intensity of 86 dB and a stimulus onset asynchrony (SOA) of 30, 60, 120, and 240 ms, resulting in four prepulse (PP) trial conditions. Rise and fall times of all stimuli were less than 1 ms. Four startle stimuli were presented at the beginning (block 1) and four startle stimuli at the end (block 4) to assess habituation. Eight no-stimulation (NS) trials and eight prepulse-alone (PPA) trials were recorded to assess baseline EMG activity. PPA trials, NS trials and each of 4 prepulse trial conditions were presented in a pseudorandomized order in blocks 2 and 3.

Supplemental Results

Cannabis and MDMA

Groups of high and low cannabis and MDMA users were created by median split of cumulative use. Cannabis use (cumulative use, duration of use, grams per week) was not correlated with %PPI in the cocaine user group. An analysis of covariance comparing the % prepulse inhibition (%PPI) mean across conditions between cocaine users with no (n = 9), low (n = 41), and high (n = 41) cannabis use did not reveal a significant difference (F(2,85) = .72, p = .49) (Figure S6). Introducing cumulative cannabis use as a covariate in the main PPI analysis did not alter the results.

MDMA use measures (cumulative use, duration of use, tablets per week) were also not correlated with %PPI in the cocaine user group (all p > .31). The comparison of %PPI mean across conditions between cocaine users with no (n = 26), low (n = 37), and high (n = 30) MDMA use did not reveal a significant difference (F(2,87) = .20, p = .82) (Figure S7). Moreover, introducing cumulative MDMA use as a covariate in the main PPI analysis did not change the results.

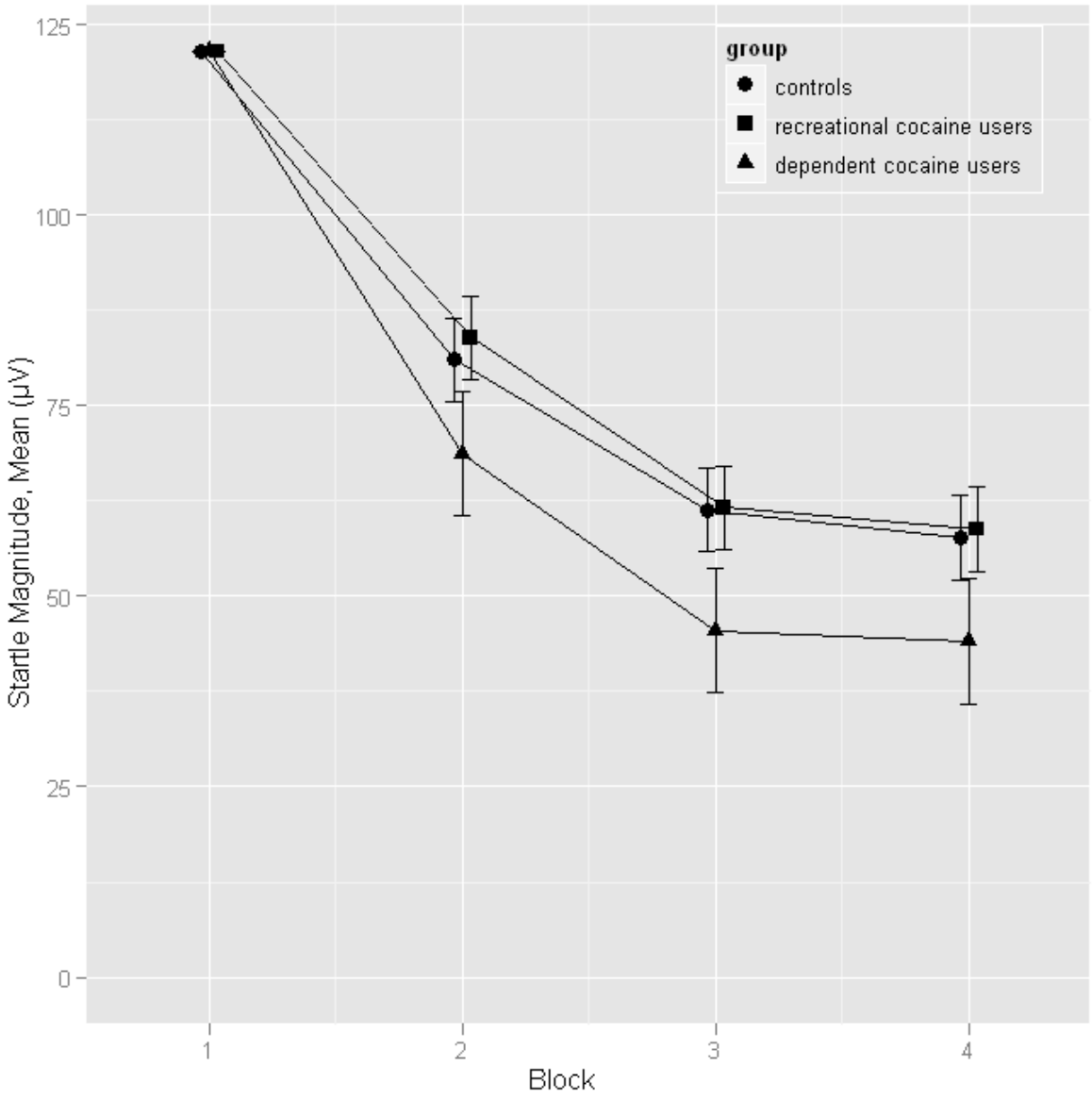


Figure S1. Habituation curve diagrammed as mean amplitude of pulse-alone (PA) trials in four blocks, corrected for startle reactivity in the first block (means ± SEM) for recreational (n = 64) and dependent cocaine users (n = 29), and healthy control participants (n = 66). Each block contains four 116-db PA trials.

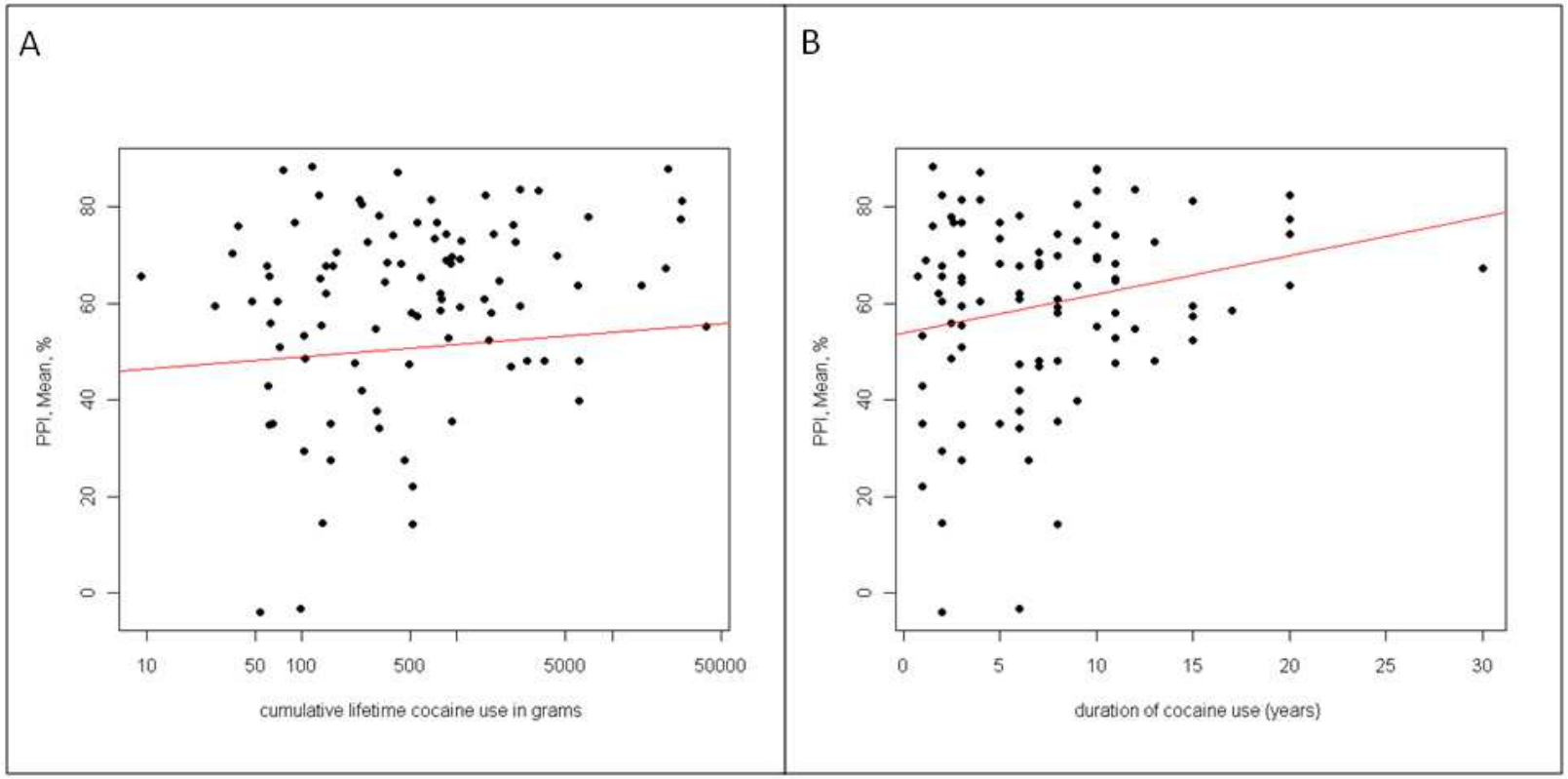


Figure S2. Cumulative lifetime cocaine use in grams (ln-transformed) and mean % prepulse inhibition (%PPI) across all conditions in cocaine users ($r = .23$, $p < .05$) (**A**) and duration of cocaine use in years and mean %PPI across all conditions in cocaine users ($r = .22$, $p < .05$) (**B**). The exclusion of the outlying value reporting 30 years of cocaine use did not change the correlation between duration of cocaine use and %PPI ($r = .23$, $p < .03$).

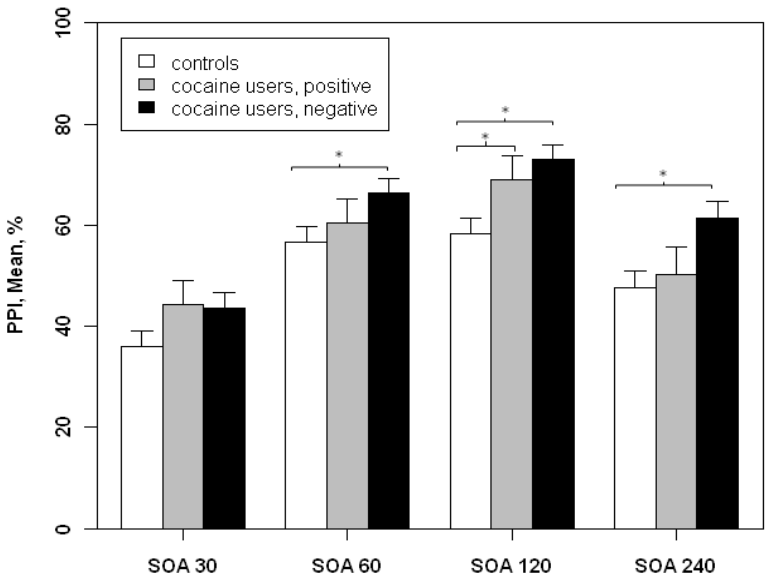


Figure S3. Mean % prepulse inhibition (%PPI) in cocaine users with positive (n = 26) and negative (n = 67) urine samples for cocaine, and healthy control participants (n = 66). Error bars refer to SEM. *indicates significant difference between groups (Sidak-post hoc test: $p < .05$). SOA, stimulus onset asynchrony.

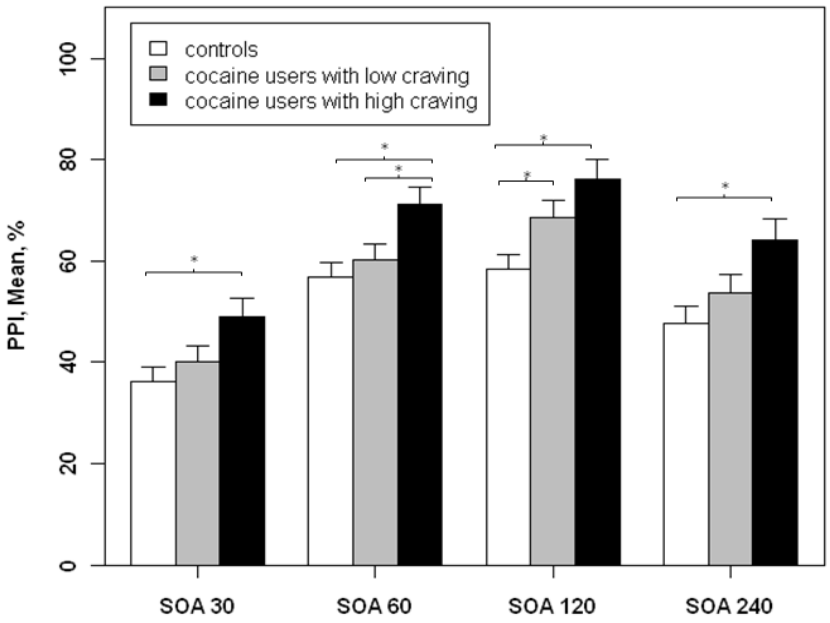


Figure S4. Mean % prepulse inhibition (%PPI) in low (n = 52) and high (n = 41) craving cocaine users and controls (n = 66). Cocaine users with high craving show increased PPI. Error bars refer to SEM. *indicates significant difference between groups (Sidak-post hoc test: $p < .05$). SOA, stimulus onset asynchrony.

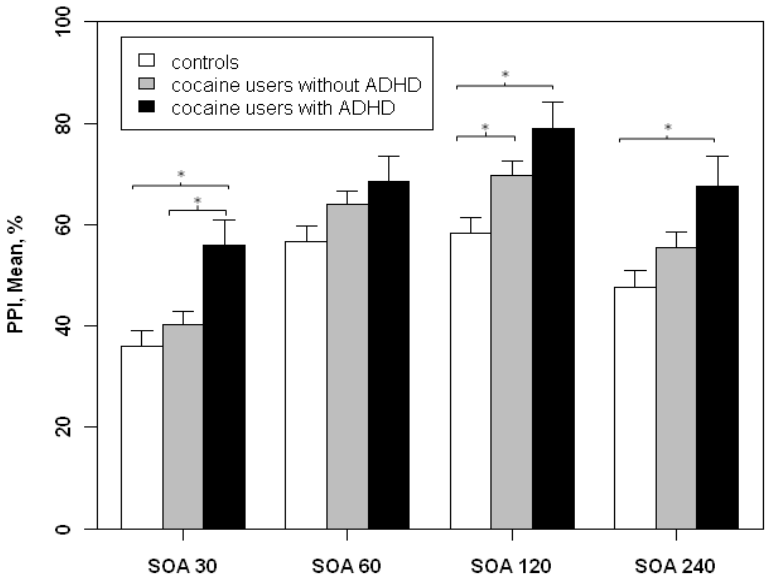


Figure S5. Mean % prepulse inhibition (%PPI) in cocaine users with attention-deficit/hyperactivity disorder (ADHD) (n = 22) and without ADHD (n = 72), and healthy control participants (n = 66). Error bars refer to SEM. *indicates significant difference between groups (Sidak-post hoc test: $p <$ .05). SOA, stimulus onset asynchrony.

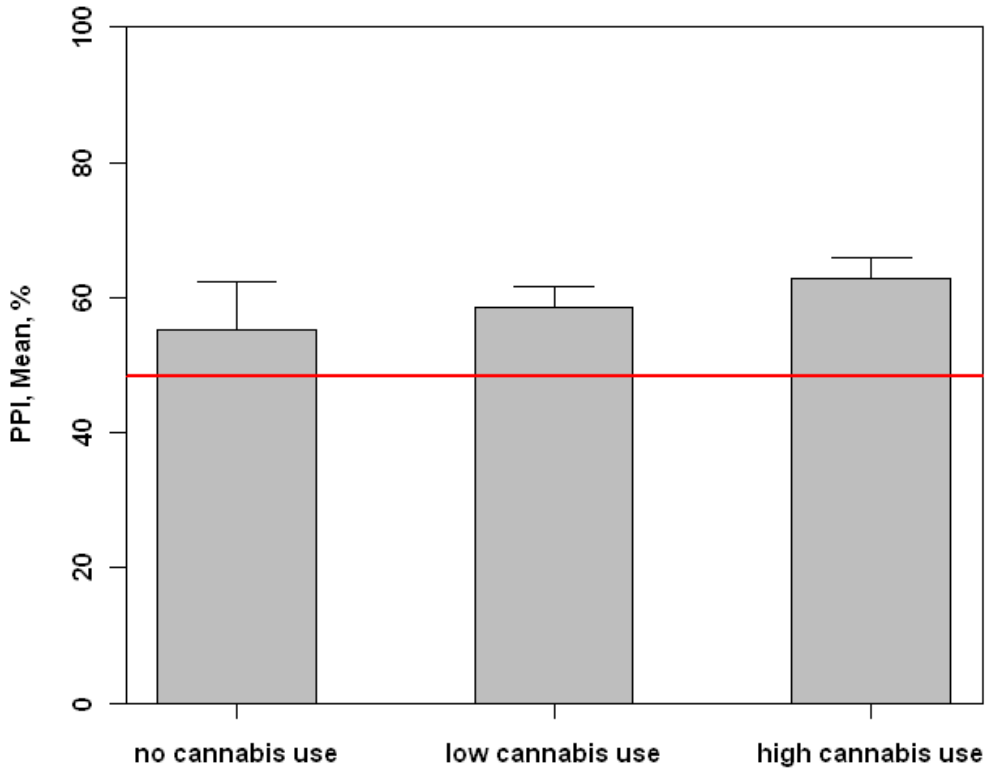


Figure S6. Mean % prepulse inhibition (%PPI) across conditions in cocaine users with no (n = 9), low (n = 41) and high (n = 41) cumulative cannabis use. The red line represents the PPI level of the controls without cannabis use for comparison. Error bars refer to SEM.

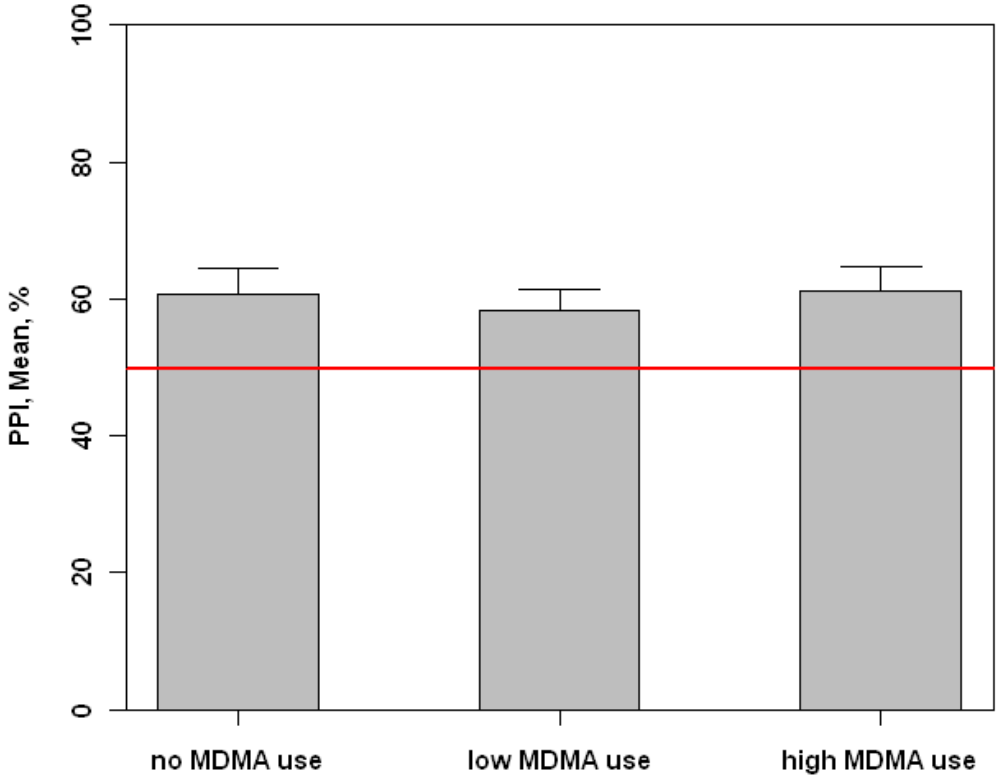


Figure S7: Mean % prepulse inhibition (%PPI) across conditions in cocaine users with no (n = 26), low (n = 37) and high (n = 30) cumulative MDMA use. The red line represents the PPI level of the controls for comparison. Error bars refer to SEM.